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The effect of different polychlorinated biphenyls on two aquatic models, the green alga *Pseudokirchneriella subcapitata* and the haemocytes from the European abalone *Haliotis tuberculata*

Marie-Pierre Halm-Lemeille [a,b,*], Elham Abbaszadeh Fard [a,b,c], Thomas Latire [a,c], Jean-François Ferard [d], Katherine Costil [a,c], Jean-Marc Lebel [a,c], Ronan Bureau [a,b], Antoine Serpentini [a,c,*]

[a] Normandie Université, F-14032 Caen, France
[b] Centre d'Etudes et de Recherche sur le Médicament de Normandie, UPRES EA-4258, INC3M FR CNRS 3038, SFR ICORE, Université de Caen Basse-Normandie, UFR des Sciences pharmaceutiques, Boulevard Becquerel, F-14032 Caen cedex, France
[c] CNRS INEE, FRE3484 BioMEA, SFR ICORE, IBFA Université de Caen Basse-Normandie, IBFA, Esplanade de la Paix, F-14032 Caen, France
[d] Université de Lorraine, Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC), UMR 7360 CNRS, Rue du Général Delestraint, F-57070 Metz, France

HIGHLIGHTS

- Comparison of ecotoxicity of PCBs on a green algae and the haemocytes from gastropod.
- Green algae median EC_{50} values ranged from 0.34 μM to more than 100 μM.
- Abalone EC_{50} values ranged from 1.67 μM for PCB153 to 89 μM for PCB28.
- No differences between the DL and NDL PCBs ecotoxicities regardless of the model used.
- Our results demonstrated that the ecotoxicities of PCBs were model dependent.

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ABSTRACT

The present study was conducted to determine the toxicity of different polychlorinated biphenyls (PCBs) on the green algae, *Pseudokirchneriella subcapitata* and the haemocytes from the European abalone, *Haliotis tuberculata*. Using the algal growth inhibition test, the green algae median Effective Concentration (EC_{50}) values ranged from 0.34 μM for PCB28 to more than 100 μM for PCBs 101 and 153. Considering the MTT viability test, the abalone EC_{50} values ranged from 1.67 μM for PCB153 to 89 μM for PCB28. Our results in contrast to previous observation in vertebrates did not show significant differences between the dioxin like- and non dioxin like-PCBs toxicities regardless of the model used. However, our results demonstrated that the toxicities of PCBs were species dependent. For example, PCB28 was the most toxic compound for *P. subcapitata* whereas PCBs 1, 180 and 153 were less toxic for that species. On the contrary, PCB153 was reported as the most toxic for *H. tuberculata* haemocytes and PCB28 the least toxic. To investigate the mode of action of these compounds, we used an *in silico* method. Our results suggested that PCBs have a non-specific mode of action (e.g., narcosis) on green algae, and another mode of action, probably more specific than narcosis, was reported for PCBs on the abalone haemocytes.

1. Introduction

For the past several decades, human activities have discharged large quantities of environmental pollutants. Some of the pollutants introduced into terrestrial and aquatic ecosystems may possess toxic effects. Some of them belong to persistent organic pollutants (POPs) which are organic compounds that are resistant to biodegradation. As a consequence, these molecules persist in the environment, bioaccumulate in the biota and in some case are magnified through the food chain (Connolly, 1991; Crinnion, 2011a).

Among POPs, polychlorobiphenyls (PCBs) are compounds of anthropogenic origin that possess 209 congeners differing in the number and position of chlorine atoms. Due to their physical

* Corresponding authors. Address: Normandie Université, F-14032 Caen, France. Tel.: +33 2 31 56 68 23; fax: +33 2 31 56 59 10 (M.-P. Halm-Lemeille). Tel.: +33 2 31 56 56 80; fax: +33 2 31 56 53 46 (A. Serpentini).
E-mail addresses: marie-pierre.halm@unicaen.fr (M.-P. Halm-Lemeille), antoine.serpentini@unicaen.fr (A. Serpentini).

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chemicals properties, PCBs were used as insulators in transformers, hydraulic fluids, paint additives, fire retardants, and pesticide extenders (Eisler and Belisle, 1996; Erickson and Kaley, 2011). Although their production ended in the late 1980s, they are still present in the environment and, thus, can give rise to ecological risks resulting in a close monitoring of these contaminants worldwide (Jensen et al., 1969; Howell et al., 2008; Carvalho et al., 2009; Hauck et al., 2010; Montory et al., 2011). Although the concentrations of these compounds in the environment have been declining, they are still listed as Priority Substances within the EU Water Framework Directive (Directive 2013/39/EU, 2013). These pollutants are reported to cause detrimental effects to wildlife and humans, including carcinogenesis, mutagenesis, endocrine disruption, and immunotoxicity (Safe, 1984; Eisler and Belisle, 1996; ATSDR, 2000; Crinnion, 2011b).

Due to their (eco)toxicological effects and their magnification along food chains, they still pose a serious environmental concern (Sinkkonen and Paasivirta, 2000; Beyer and Biziuk, 2009). For example, many studies have shown the accumulation ability of PCBs by phytoplankton, which are implicated in food web alteration and resultant damages to commercially important fisheries (O'Connors et al., 1978).

In Europe, seven PCBs were chosen by the European Commission as being of PCB research and study primarily because of their abundance in the environment and their toxicological properties (McFarland and Clarke, 1989). The impacts of PCBs on the environment and biota are due to the individual components of this mixture and their additive and/or nonadditive interactions among themselves and other chemical classes of pollutants (Giesy and Kannan, 1998). It has been shown that congener profiles of PCBs in environmental media can be changed significantly from the profiles of commercial mixtures (e.g. Aroclor). Therefore, risk assessment based on commercial mixture may not be representative of the risks posed by PCBs (WHO, 2001). Several studies have demonstrated differences in both mechanisms and toxic potentials of individual PCB congeners (Henry and DeVito, 2003). Thus, the hazard assessments of PCBs require ecotoxicological studies on individual PCB congeners (WHO, 2001).

The interactions of PCBs with the biota or properties of individual congeners like bioaccumulation, binding to biological receptors (Boon et al., 1997), toxicity (Eriksson et al., 2002) or metabolism (Yunker et al., 2011) depend upon both the number of chlorines and their position around the biphenyls rings. For many years, concerns of PCB toxicity have focused on dioxin-like actions (immunosuppression and carcinogenesis) mediated via activation of the aryl hydrocarbon receptor and the induction of cytochrome P450 (Safe, 1999, for revue). These effects are primarily due to congeners that have chlorines only in the *meta* and *para* positions which can assume a coplanar dioxin-like configuration. Congeners with chlorines in the *ortho* position are energetically dissuaded from assuming a coplanar configuration. Because of this diversity in biological activities, PCBs show a wide range of potential effects (Fisher et al., 1998; ATSDR, 2000; Henry and DeVito, 2003). Although the mechanisms of action of PCBs are relatively well known in vertebrates, the biological effects and the mechanisms of action of PCBs are not yet completely understood in invertebrate organisms or phytoplankton (WHO, 2001).

The purpose of this study is to generate new ecotoxicologic data of individual PCBs based on their structural properties on phytoplankton and invertebrate and to propose a possible interpretation in terms of mode of action. Phytoplankton forms the basis of many aquatic food chains. Most studies on the impact of PCBs on phytoplankton focuses on bioaccumulation capacities and the knowledge concerning their toxicity is in state confusing (e.g. photosynthetic mechanism, chloroplast alteration) (Conner and Mahanty, 1979). In the present study, we have chosen to use the green algae *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*) as a biological model. This alga is commonly used as a model for standard toxicity tests (OECD, 2006; US-EPA, 2002; ISO 8692, 2004). Its use is widespread in ecotoxicological tests mainly because of its ease of cultivation, rapid growth (Nygaard et al., 1986) and sensitivity to a wide range of contaminants (Kamaya et al., 2003; Colombo et al., 2008; Aruoja et al., 2009; Pretti et al., 2009; Wik et al., 2009). *P. subcapitata* is an excellent indicator organism of pollution in aquatic ecosystems where trace contaminants are difficult to analyse directly. However, to our knowledge, few studies exist in the literature on the effect of PCBs on these green algae. For example, results obtained by Mayer et al. (1998) indicated the effective concentration (EC_{50}) of three PCBs (PCB31, 48 and 105) after 48 h or 72 h of exposure. These authors calculated EC_{50} values of 241 nM and 14 nM for PCB31 and 105, respectively, after 24 h exposure. For PCB48, an EC_{50} value of 134 nM was obtained after an exposure of 72 h.

Additionally, we used a non-conventional model to compare the effects of PCBs: the haemocytes from the European abalone, *Haliotis tuberculata*. Haemocytes are cells that are continually exposed to the external environment due to the open circulatory system of molluscs. To assess the effects of PCBs on the abalone immune response, an *in vitro* approach was chosen. *In vitro* approaches are alternative experimental methods to whole- animal testing that are employed because of the reduced use of animals, capability for standardisation, low cost and rapid performance associated with these methods, in addition to ethical considerations (Schirmer, 2006; Shuilleabhain et al., 2006). Although *in vitro* assays may not always reflect the true *in vivo* situation, which is more complex, they undoubtedly provide important data to determine mechanisms at the molecular and the cellular levels (Binelli et al., 2009). Indeed, cell cultures have allowed cells to be studied in a controlled environment and in isolation from the multiple physiological systems that regulate their activities *in vivo*. It is for this reason that marine invertebrate cell cultures had been developed during the last decade to study physiological processes (Brousseau et al., 2000; Olabarrieta et al., 2001; Canesi et al., 2003; Gagnaire et al., 2006; Duchemin et al., 2008; Mottin et al., 2010; Latire et al., 2012). However, few investigations have reported the effects of PCBs on marine molluscs at the cellular level. For example, Gagnaire et al. (2006) have investigated the *in vitro* effects of PCB77 and 153 on the haemocytes from the oyster *Crassostrea gigas*. These authors demonstrated that PCB 77 induced a significant decrease of lysosome-positive haemocyte percentage after an incubation periods of 4 h at 6 and 60 μM., but no effects were recorded on other tested parameters (e.g. cell viability, phagocytose activity, ROS production). However PCB153 had no effects suggesting that the effects of PCBs to be congener-specific in mollusc. In mussel haemocytes (Canesi et al., 2003), PCB47 and 153 can alter immune parameters (e.g. microbial activity and lysosomal enzyme release, respectively). Additionally, both PCBs and PCB77 reduced hemocyte lysosomal membrane stability. Moreover these PCBs act on molecular targets involved in signal transduction (e.g. tyrosine kinase-mediated cell signalling) corresponding to those found in human neutrophils (Canesi et al., 2003). These results suggest that haemocytes represent a useful model for evaluating the potential effect of PCB.

In order to assess the toxic properties of different congeners, we chose representative PCBs among the 209 congeners according the number of chlorines and their position around the biphenyls rings and their potential toxicity as a function of environmental frequency (Rein and Bittens, 2004). In their study, Rein and Bittens defined 4 groups of PCBs: "highest toxic potential" (group A), "high to moderate toxic potential" (group B), "low toxic potential" (group C) and "no toxic potential reported" (group D). In our study, we analyse the effects of PCBs belonging to each group (PCB77,